RNA surveillance down-regulates expression of nonfunctional κ alleles and detects premature termination within the last κ exon

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Random V(D)J junctions would generate nonfunctional and/or out-of-frame sequences in about two-thirds of cases and result in abundant transcripts encoding truncated proteins. Although allelic exclusion at the DNA recombination level ensures that a single allele is functional, the frequent biallelic rearrangements need additional mechanisms to down-regulate aberrant transcripts in those cells with both a functionally and a nonfunctionally rearranged allele. The process of nonsense-mediated decay targets aberrantly rearranged Ig heavy-chain transcripts, but the situation of light-chain mRNAs is more complex, because they do not meet the usual requirements for nonsense-mediated decay and most often lack a spliceable intron downstream of the premature termination. We studied immunoglobulin heavy-chain −/− pro-B cells in which light chain genes get rearranged and expressed in the absence of any selection for the assembly of a functional B cell receptor. Using this model, we show that the whole κ locus is accessible in pro-B cells and allows the assembly of a broad spectrum of VκJκ segments, most of which are out-of-frame. This model provides an evaluation of the *in vivo* efficiency of RNA surveillance toward aberrant κ mRNAs produced in pro-B cells. Our data show that nonfunctional κ transcripts are excluded from the mature mRNA pool not only by detecting termination in an upstream exon but also by detecting changes in the position of termination within the last exon. Similar mechanisms efficiently down-regulate nonfunctional κ transcripts arising in normal mature B cells due to the biallelic transcription of rearranged κ genes.

Random deletions or additions at the Ig V(D)J junctions generate two-thirds of nonfunctional out-of-frame rearranged genes in B cell progenitors (1–3). Cell selection further allows the outgrowth of clones, each producing a single Ig selected for functionality and affinity for a non-self antigen determinant (4). Although some of those clones carry both functional and nonfunctional alleles of the immunoglobulin heavy-chain (IgH) and/or light IgL loci, expression of truncated proteins is not observed in normal B cells, where aberrant transcripts are only present in very low amounts (5). For κ light chain (LC) genes, although the observed frequency of B cells carrying both a functional and a nonfunctional allele is >30% (1, 3), out-of-frame κ mature mRNA sequences were rarely obtained (6). Such an exclusion of nonfunctional Ig mRNA in B cells could involve transcriptional down-regulation and/or mRNA quality control processes.

Allelic asymmetry at the transcriptional level has been demonstrated for several cytokine genes (7, 8). Concerning Ig genes, differences in the methylation status of alleles have been reported and correlated with an asymmetry in the replication pattern and the nuclear localization (9–10). However, transcriptional silencing of an excluded Ig gene allele has not been demonstrated, and, on the contrary, detection of rare out-of-frame transcripts in normal B cells indicates that this silencing is at the most incomplete. Allelic asymmetry of the chromatin structure is established very early [before the onset of V(D)J recombination] and is likely responsible for the preferential monoallelic primary and secondary rearrangements, whereas its effect with regard to transcription of rearranged alleles is unclear (9–12). This asymmetry and the preferential use of one allele account for the predominance of cells with a single κ rearranged allele among normal B cells (1, 11). However, for the numerous cells carrying one functional and one nonfunctional rearranged κ allele, additional processes are clearly needed to explain the low frequency of sterile Ig transcripts (5).

The general pathway of nonsense-mediated decay (NMD) involves the Up-Frameshift (UPF) complex, which binds primary mRNA in the nucleus and is displaced after translocation to the cytoplasm, provided that a correct coding sequence allows active translation (13–16). By contrast, a premature termination codon (PTC) within the mature mRNA can prevent its release from UPF1 binding and drive it into a degradation pathway. A widely accepted concept is that normal mRNA with termination within the last exon is stable and actively translated, whereas NMD targets transcripts including a PTC within an upstream exon. In addition, NMD usually requires termination to occur at least 50 nt upstream of the next exon–exon junction (14, 17). Indeed in mammals, exon–exon junctions are "marked" by a multiprotein complex deposited on mRNA by the spliceosome and able to trigger NMD (18–20).

Ig LC genes provide unique models for the study of RNA surveillance pathways, because frameshifted V_L–J_L junctions most often create a PTC within the constant sequence, which (in contrast to all other receptor genes) is encoded by a single 3′-terminal exon. Previous studies focused on the situation created by somatic hypermutation and the random generation of a stop codon within the V exon (5, 21). By contrast, the frequent occurrence of out-of-frame V–J junctions may have been overlooked. In that case, a frameshift will either cause the occurrence of a PTC within the last exon encoding the C domain or will less frequently create a PTC within the penultimate exon, at only three nucleotides from the exon–exon junction. Out-of-frame κ transcripts thus do not in theory meet the requirements of NMD. The study of RNA surveillance with regard to Ig LC should thus help in unraveling the molecular features of the additional down-regulatory pathways that potentially eliminate aberrant transcripts encoding truncated proteins (22).

To get a clearer picture of RNA surveillance efficiency, we determined the frequencies of in-frame vs. out-of-frame κ transcripts in the absence of any requirement for a functional κ LC. Pro-B cells from mice with a disrupted IgH locus were studied, excluding processes that may have been operating at the cell selection level and that may have favored those cells with a down-regulated expression of nonfunctional genes. Random κ junctions accumulated freely in such cells blocked at the pro-B cell stage and provided an opportunity to study the relative representation of in-frame or out-of-frame sequences among primary and

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Abbreviations: CDR, complementarity determining region; F, functional; IgH, immunoglobulin heavy chain; LC, light chain; NF, nonfunctional; NMD, nonsense-mediated decay; PTC, premature termination codon.

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mature κ transcripts. In parallel, we also studied the expression of out-of-frame κ junctions corresponding to nonfunctionally rearranged and "excluded" genes in normal B cells.

Materials and Methods

Animals, Cells, and Cultures. IgH−/− mice carrying a *neo* insertion between JH and Eμ have been described (23). Insertion of the *neo* gene resulted in a complete lack of mature IgH transcripts and in a blockade of B cell maturation at the pro-B stage. Mice were housed under conventional conditions according to principles of animal care and were killed with carbon dioxide according to recommendations of the regional ethics committee.

Bone marrow cells and splenocytes were collected from 5-week-old mice. Splenocytes were labeled with either FITC-labeled anti-CD19 or biotin-labeled anti-κ antibodies (Southern Biotechnology Associates), incubated with anti-biotin microbeads, and sorted on an Automacs apparatus (Miltenyi Biotec, Bergish Gladbach, Germany). Cells were either directly used for nucleic acid preparation or cultured for 4 h in the absence or presence of 100 μg/ml cycloheximide (Sigma) as shown to result in a >95% inhibition of protein synthesis (24). Cells grown in parallel were labeled for 4 h in media containing 50 μCi/ml (1 Ci = 37 GBq) Expre^{35}S^{35}S labeling mix (methionine 77%, cystein 17%; Perkin–Elmer) and lysed in 0.5% Nonidet P-40 buffer. Labeled Ig were adsorbed on protein G beads (Amersham Biosciences) and separated on polyacrylamide gels.

RNA Preparation and RT-PCR. RNA was prepared in TriPure (Roche Applied Science). RT-PCR was carried out on DNase I-treated (Invitrogen) RNA and checked to be negative in the absence of RT, ruling out contamination by genomic DNA. RT was carried out for 1 h with Superscript II (Invitrogen) from 1 μg of total RNA, either with random hexamers for the study of mature transcripts or with a primer located in the Jκ–Cκ intron downstream of the RS element for the study of primary transcripts from Vκ–Jκ segments associated to Cκ (3′-RS primer: 5′-CAGATTAGTGGCTCTGTTCCT-3′).

For PCR amplification of mature transcripts, primers were a forward Vκ consensus primer (25) [5′-CAG(G/C)TTCAGTG-GCAGTGG(A/G)TC(A/T)GG(A/G)AC-3′] and a backward primer complementary to the 5′ end of the murine Cκ exon [5′-GCACCTCCAGATGGTTAACTGC-3′]. The PCR products with an expected size of ≈210 bp were analyzed on 2% agarose gels.

For PCR amplification of primary transcripts, backward primers were chosen within the introns immediately after Jκ1 (5′-CACAGACATAGACAACGG-3′; expected size ≈180 bp), Jκ2 (5′-CTTAGTGAACAAGAGTTGAGAAGACATC-3′; expected size ≈170 bp), and Jκ5 (5′-CTCAAGATTTTCTGAACT-GAC-3′; expected size ≈260 bp).

DNA Cloning and Sequencing. DNA was prepared with proteinase K (Roche Applied Science) and PCR-amplified with the forward Vκ consensus primer and the reverse Jκ1 or Jκ5 primers.

All PCR and RT-PCR products were cloned into pCRII TOPO vector (Invitrogen). DNA sequences were run on an automated sequencer (Applied Biosystems) and compared with those from GenBank by using the BLAST algorithm (26) (www.ncbi.nlm.nih.gov). Comparisons with germ-line κ genes followed the nomenclature of H. G. Zachau (27–29). Analysis of V–J junctions provided the ratio of nonfunctional/functional sequences (NF/F ratio).

Complementarity Determining Region (CDR) 3 Length Analysis. Lengths of the bulk of V–J junctions from primary cells were estimated through capillary electrophoresis analysis of amplified products encompassing the Vκ–Jκ junction (with PCR and RT-PCR products obtained with a fluorescently labeled consensus Vκ primer) by using GENESCAN software on a capillary 3100 sequencer (Applied Biosystems). Computing of electrophoresis data provided the areas of the peaks corresponding to each CDR3 length and allowed calculation for each sample of the NF/F ratio.

Results

Features of κ Sequences Expressed in Pro-B Cells. We studied mature κ mRNAs present in pro-B cells from IgH−/− mice. These cells do undergo VJ rearrangement of the κ locus, at a frequency corresponding to the low-level κ rearrangements that may precede IgH rearrangements in normal B cells (30–32). Mature κ transcripts were obtained (89 independent junctions), which identified >30 different Vκ genes joined to all four functional Jκ segments (Fig. 1). This pro-B repertoire involved Vκ segments dispersed throughout the κ locus (including the most upstream hf24) and belonging to the four Vκ contigs (Table 1) (27–29). No transcript included the Jκ3 segment known to lack a functional recombination signal (33).

VκJκ sequences from IgH−/− mice were heterogeneous: the CDR3 segment varied from 15 to 37 nt (mean 27.4 ± 2.74), whereas the canonical length of 27 nt/9 codons was only found in 27% of cases (Fig. 1). In addition, junctions showed nucleotide (N) insertions whose lengths approached those of $V_H(D)J_H$ insertions (mean length of N insertions within IgH genes 2.86 ± 2.05, mean length within pro-B κ junctions 1.69 ± 1.54) (23) (Fig. 1).

By contrast, κ sequences from mature B cells have homogeneous CDR3 lengths because of the requirements of pairing with a functional heavy chain, and 90% of normal κ chains have a nine-codon CDR3 (34). As controls, we determined Vκ–Jκ junctions from 59 independent cDNA clones from WT mice splenocytes. All were in-frame, and 55 (93.2%) had a nine-codon CDR3 (two were 30 nt and two were 24 nt, and the mean was 27 ± 0.36). N insertion was absent in 57 of 59 control sequences, so that the mean insertion length was 0.07 ± 0.13.

Down-Regulation of Nonfunctional κ Rearrangement Expression in Pro-B Cells. In the absence of any selection for a functional B cell receptor, IgH−/− pro-B cells are expected to randomly rearrange their κ locus, so that nonfunctional junctions should represent about twice the number of functional sequences (1, 2). By sequencing randomly selected rearranged VκJκ genes from genomic DNA, we indeed found in such cells that the ratio of NF/F genes was 1.77, because 23 of 36 sequences analyzed were nonfunctional (11 had a +1 frameshift, and 12 had a +2 frameshift). On the contrary, when cDNA sequences from the same cells were analyzed, the NF/F ratio fell to 0.48 (Fig. 1 and Table 2). Such a difference indicated that expression of nonfunctional sequences was reduced by ≈4-fold in pro-B cells in comparison with fully translatable sequences that do not undergo NMD.

Nonfunctional sequences could be analyzed with regard to the position of the PTC in either the last or the penultimate exon of the transcript, because only the latter position should meet the usual requirements of NMD. The situation of VκJκ1 rearrangements is peculiar, because Jκ1 is translatable in all three reading frames, and any out-of-frame mRNA terminates within the last exon. Nevertheless, a >4-fold down-modulation of out-of-frame transcripts did involve VκJκ1 joints so that only 8 of 29 (NF/F ratio 0.38) were out-of-frame (Fig. 1). Altogether, analysis of nonfunctional κ cDNA sequences allowed the delineation of two main classes (Fig. 2): (*i*) The C^{PTC} class included sequences with a PTC within the last (Cκ) exon, i.e., any out-of-frame Jκ1 rearrangement and those Jκ2, Jκ4, or Jκ5 rearrangements with a +2 frameshift. Within the C^{PTC} class, a +2 frameshift in any Jκ causes termination at codon +2 of the Cκ, whereas a +1 frameshift in Jκ1 causes termination at codon +18 of Cκ. (*ii*) The VJ^{PTC} class included transcripts with termination within the penultimate exon, i.e., Jκ2, Jκ4, and Jκ5 rearrangements with a +1 frameshift (creating a PTC at only 3 nt upstream from the splice junction) and rearrangements with a stop codon created at the V–J junction.

Assuming that all functional κ transcripts have similar half-lives, the proportion of rearrangements on the Jκ1, Jκ2, Jκ4, and Jκ5

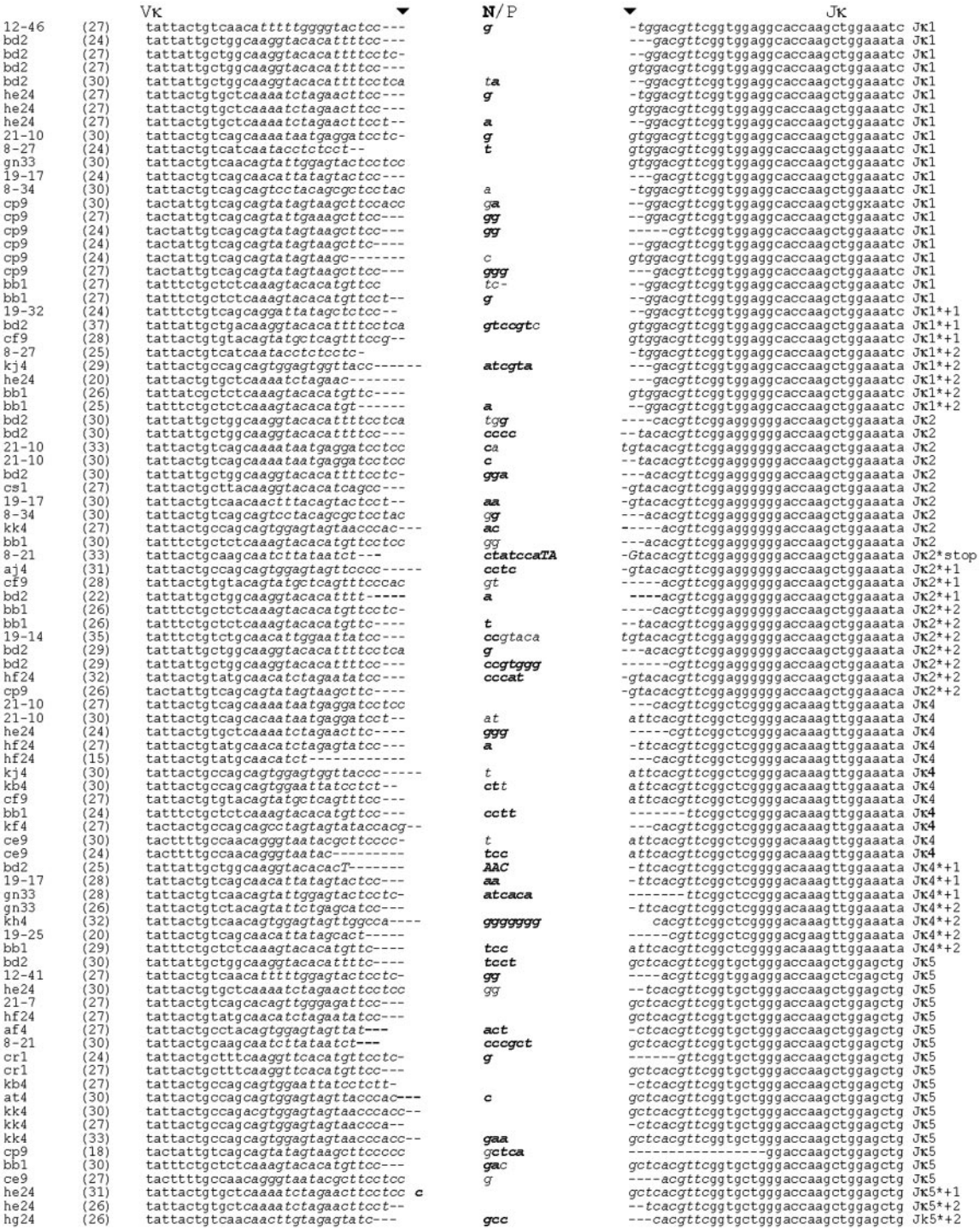

Fig. 1. Vκ–Jκ junctions in bone marrow pro-B cells from N/N homozygous mice. Sequences are given with reference to the name of the corresponding germ-line Vκ and Jκ segment. Dashes indicate junctional deletions; N insertions (bold) and P insertions are indicated in between. Italicized nucleotides encode the CDR3 segment (whose length is indicated in parentheses); nonfunctional sequences are marked by an asterisk with indication of the +1 or +2 frameshift or of an in-frame stop codon (in upper case).

segments can be deduced from the relative frequencies of the corresponding in-frame κ transcripts in pro-B cells, i.e., 35, 16.7, 20, and 28.3%, respectively. Concerning out-of-frame rearrangements at the DNA level, those corresponding to the VJ^{PTC} group would thus be expected to be ≈32.5% (one of two out-of-frame rearrangements involving Jκ2, Jκ4, or Jκ5), and the remainder (corresponding to the C^{PTC} group) would be expected to be 67.5%. The observed proportions of mature out-of-frame transcripts did not significantly differ from these estimations and were in fact 28% (8 of 29) and 72% (21 of 29), respectively, for the VJ^{PTC} and C^{PTC} classes. Both classes of PTC-bearing transcripts thus appeared to undergo a similar level of down-regulation (Table 2).

By contrast, significant differences (χ^2 test, $P < 0.01$) appeared when the proportion of functional vs. nonfunctional κ mature

Table 1. Vκ usage in pro-B cell stage junctions

Contig	Gene segment	Jκ1	Jκ2	Jκ4	Jκ5
Z4–1	hf-24		**1**	**2**	**1**
	bd2	**5**, 1	**6**, 1	**1**	**1**, 5
Z4–5	kb4	5		**1**	**1**, 2
Z4–7	cr1				**2**
Z4–8	he24	**4**		**1**	**3**
	bb1	**4**	**3**	**2**	**1**, 1
	hg24	1	1		**1**
Z4–11	cf9	**1**	**1**		**1**
Z4–12	ce9	2	1	**2**	**1**
Z4–13	kf4		1	**1**	1
	cp9	**6**, 1	**1**, 1		**1**, 2
Z4–14	aj4		**1**		
	cs1		**1**		
Z4–15	gn33	**1**		**2**	
Z4–16	af4				1
Z4–19	km4	2	1		
Z3	kk4		**1**, 1		3, 1
	kj4	**1**		**1**	1
Z2	at4				**1**
	kh4			**1**	
Z1–Z2	ko4		2		
Z1–9	12–46	**1**			
Z1–8	12–41				**1**
Z1–6	19–32	**1**			
Z1–5	8–27	**2**, 1			
	8–34	**1**	**1**		
Z1–4	19–25			**1**	
Z1–3	8–21	1	**1**		**1**
	19–17	**1**	**1**	**2**	
	19–14		**1**		
Z1–2	21–10	**1**	**2**	**2**	1
Z1–1	21–7				**1**

RNA junctions are in bold.

transcripts were analyzed according to the rearranged Jκ segment and when Jκ2 or Jκ4 was compared with Jκ1 or Jκ5 (Table 2). RNA surveillance appeared to be less efficient for VκJκ2 and VκJκ4 (NF/F ratio of 1.1 and 0.58, respectively). By contrast, down-modulation of nonfunctional sequences was stronger for VκJκ5 and VκJκ1 transcripts (NF/F ratio of 0.18 and 0.38, respectively), although the latter all belonged to the C^{PTC} class.

Nonfunctional κ Junctions Are Detected as Unspliced Transcripts in both Blocked Pro-B Cells and WT B Cells. Capillary electrophoresis appreciated the relative amounts of each CDR3 length and allowed

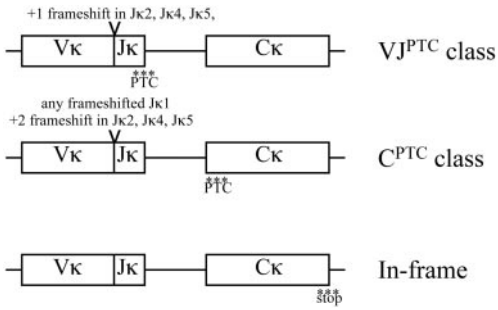

Fig. 2. Schematic structure of the various classes of Vκ–Jκ rearrangements. Positions of the PTC resulting from a frameshifted rearrangement and of the normal in-frame stop codon are indicated (***).

their comparison at the DNA level and among primary or mature transcripts. Data obtained in pro-B cells about κ CDR3 lengths at the DNA and the mature mRNA levels were in complete agreement with those gained from sequence analysis, thus validating this method for the relative quantification of CDR3 lengths and for the evaluation of NF/F ratios (Fig. 3 *A Right* and *C Right*): pro-B cells showed CDR3-length heterogeneity and a high proportion of out-of-frame junctions at the DNA level (NF/F ratio 2.02 ± 0.49) and at the primary RNA level (NF/F ratio 1.53 ± 0.03). By contrast, frameshifted mature transcripts were down-regulated, and, although heterogeneous in length, a majority of mRNAs were in-frame (NF/F ratio 0.39 ± 0.05). That out-of-frame junctions were abundant among primary transcripts confirmed that all types of junctions were transcribed and that the decay of nonfunctional sequences was restricted to the mature mRNA compartment.

In the WT bone marrow or spleen samples (including cells at various B lineage maturation stages), the nine-codon canonical CDR3 always predominated. Out-of-frame junctions were detected in DNA, whereas they were virtually absent among spliced transcripts (Fig. 3 *A Left* and *C Left*). Analysis of primary transcripts was also informative: as in blocked pro-B cells, out-of-frame junctions were clearly represented and indicated that transcription of allelically excluded rearranged κ genes occurs in normal B lineage cells (Figs. 3*B* and 4). The decreased NF/F ratio in primary RNA Vκ–Jκ1 junctions in comparison with DNA (0.32 ± 0.07 vs. 0.87 ± 0.19) is likely due to the frequent multiple κ DNA junctions detectable in normal B cells because of secondary rearrangements involving inversion of the first rearranged VκJκ1 gene (12). By contrast, when Vκ–Jκ5 junctions were analyzed in splenocytes, NF/F ratios appeared to be similar at the DNA level (0.46 ± 0.06) and the primary RNA level (0.52 ± 0.06) (Fig. 4). In all cases, the mature mRNA NF/F ratio was <0.01, i.e., at least 30-fold lower than in primary mRNA, and the efficiency of RNA surveillance

Table 2. Analysis of V–J junctions among mature κ transcripts

Jκ segment	In-frame	VJ^{PTC}	C^{PTC}	Total	Ratio of nonfunctional/ functional sequences
IgH −/− pro-B cells					
Jκ1	21	—	8	29	0.38
Jκ2	10	4	7	21	1.1
Jκ4	12	3	4	19	0.58
Jκ5	17	1	2	20	0.18
Total	60	8	21	89	0.48
IgH +/+ splenocytes					
Jκ1	25	—	0	25	0.0
Jκ2	13	0	0	13	0.0
Jκ4	9	0	0	9	0.0
Jκ5	12	0	0	12	0.0
Total	59	0	0	59	0.0

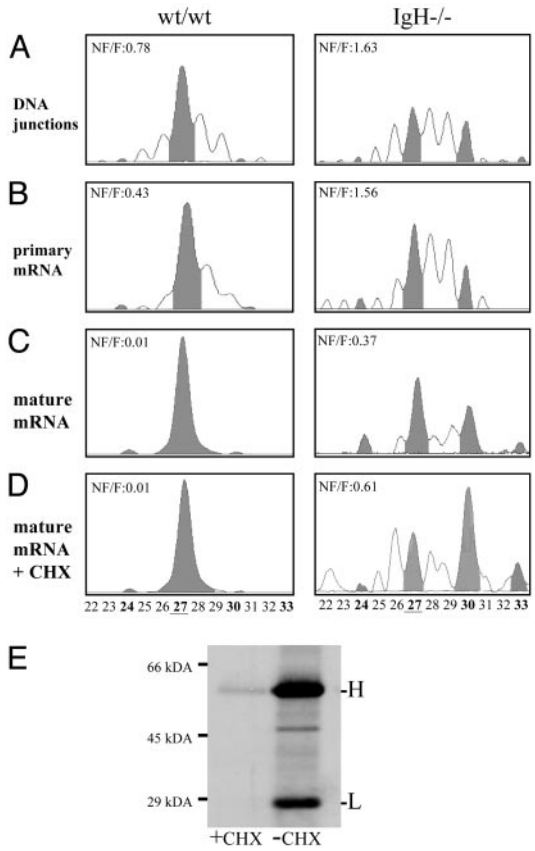

Fig. 3. CDR3 lengths and out-of-frame κ junctions. Bone marrow samples from either WT/WT or IgH−/− animals blocked at the pro-B cell stage were analyzed by high-resolution capillary electrophoresis. Data from a representative experiment are shown at the DNA level (*A*), the primary RNA level (*B*), and the mature RNA level [without cycloheximide (*C*) and with cycloheximide (*D*)]. Peaks corresponding to in-frame rearrangements are colored gray (including the dominant CDR3 with a canonical length of 27 nt); NF/F ratios are indicated. (*E*) Control of protein synthesis inhibition in cycloheximide-treated vs. untreated splenocyte cultures (metabolically labeled Ig were adsorbed on protein G and separated on a polyacrylamide gel).

thus appeared to be even higher in normal B cell populations than in pro-B cells. Similar observations were made both in bone marrow and spleen samples that included B lineage cells at various maturation stages. Some out-of-frame primary κ transcripts may arise from those λ-expressing cells that have not deleted κ genes, but they also clearly originate from the biallelic transcription of κ genes, because they were present in sorted κ-expressing cells (Fig. 4), although with a lower NF/F ratio (0.19 ± 0.02 for VκJκ5 primary transcripts) because of the selection of cells with at least one functional κ allele.

Inhibition of Protein Synthesis Partially Reverses NMD in IgH−/− Bone Marrow Pro-B Cells. Protein synthesis inhibitors are known to inhibit classical NMD (17). Because the decay of κ transcripts with aberrant V–J junctions did not apparently request the presence of a downstream intron, we wondered whether this process was also dependent on protein synthesis. We cultured for 2 to 4 h bone marrow cells from WT and IgH−/− mice in the presence or absence of cycloheximide. In the IgH−/− mice carrying numerous aberrantly rearranged κ genes, cycloheximide only yielded a partial inhibition of the decay, with an increased amount of peaks at out-of-frame positions, whereas in WT B cells it did not noticeably increased the level of out-of-frame transcripts (Fig. 3).

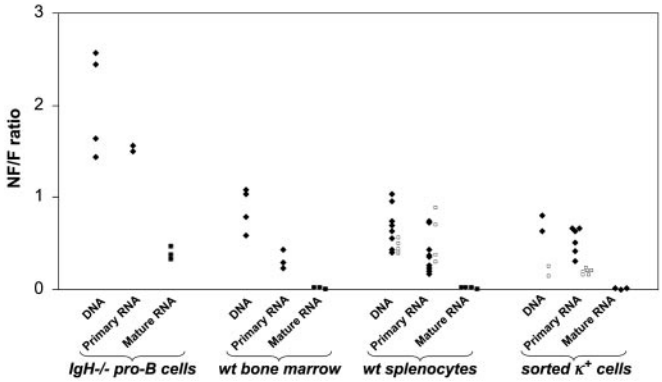

Fig. 4. Relative frequencies of out-of-frame κ junctions. NF/F ratios were estimated at the DNA, primary transcript, and mature mRNA levels by multiple experiments of capillary electrophoresis in IgH−/− pro-B cells or in tissues from normal mice (bone marrow, spleen, and κ-expressing sorted splenocytes). Filled diamonds represent values determined for Vκ–Jκ1 junctions in DNA and primary RNA and for Vκ–Jκ–Cκ junctions in mature transcripts; open squares represent Vκ–Jκ5 junctions in DNA and primary RNA.

Discussion

Low-level LC rearrangement is known to occur in cells blocked at the pro-B stage as well as in normal pro-B cells (30–32), but the repertoire and the transcription of such rearrangements were not studied. We analyzed the occurrence and the expression of VκJκ rearrangements in IgH−/− cells blocked at the pro-B stage. Mature κ transcripts were obtained from all four functional Jκ segments with a respective use of each segment that paralleled what has been previously reported in mature B cells (33, 34). A diversified and balanced Vκ repertoire was used, showing that the entire Vκ cluster is accessible to rearrangements since the pro-B stage. CDR3 lengths and V–J junctions showed a marked heterogeneity, reflecting the lack of pressure for expression of a functional κ chain pairing with a heavy chain. N insertions at V–J junctions were not significantly shorter than VH–D or D–JH junctions obtained from the IgH locus in the same cells. These insertions emphasize the normal intrinsic accessibility of κ genes to the terminal deoxynucleotidyltransferase (TdT) enzyme for early junctions occurring at the pro-B cell stage, although limited insertions occur in pre-B cells, when TdT activity is lower. N insertions stand as the hallmarks of pro-B stage junctions, but few of these rearrangements apparently allow functional Ig expression, because they are poorly represented in the mature B cell pool (2 of 59 sequences in this study). Extensively diversified Vκ–Jκ junctions with N-region additions were also reported previously in pro-B cells from μMT mice (35). In contrast, the low number of N insertions reported in κ genes from WT CD43+ cells classified as pro-B or pre-B cells (2) may emphasize a difference at the cell-selection level between late pro-B cells and transitional early pre-B cells because of the starting production of μ heavy chains (36).

Another feature of pro-B κ transcripts is the presence of out-of-frame sequences. The frequency of nonfunctional κ transcripts has not been precisely determined in normal B cells, but it is clearly very low: in the present study, all 59 control κ mRNAs from mouse splenocytes were functional. Thus, the 33% of nonfunctional pro-B cell κ mRNAs was far above what is seen in B cells. However, this frequency was still below that observed at the DNA level, with two-thirds of out-of-frame κ junctions in the absence of selection for functional B cell receptor expression.

The down-modulation of nonfunctional κ transcripts is reminiscent of the process of NMD, documented in various instances as able to differentiate normal transcripts (terminating within the last exon) from transcripts terminating in an upstream exon (13, 14).

Distance from the PTC to the next downstream exon–exon junction of the mature mRNA is usually also important; in the case of β-globin and triose phosphate isomerase genes, NMD only occurs for PTC located at least 50 nucleotides upstream from an intron, whereas, for T cell antigen receptor β transcripts, a distance of 8–10 nt may be sufficient (17, 37, 38). NMD has been studied in detail for IgH and T cell receptor β transcripts that contain multiple constant exons and that may undergo termination within the variable or the first constant exon because of frameshifted V(D)J junctions (17, 39). In a model mimicking the appearance of a PTC through somatic hypermutation right in the middle of the Vκ sequence, i.e., far upstream of the Jκ–Cκ intron, inhibition of κ transcript maturation was also demonstrated (5, 21). Contrasting in physiology, aberrant Vκ–Jκ junctions generate a majority of genes carrying a PTC within the last (and unique) Cκ exon (C^{PTC} class of transcripts), whereas those junctions terminating within the Vκ–Jκ exon (VJ^{PTC} class) usually include a PTC at only 3 nt 5′ to the splice junction. By studying pro-B cells with frequent out-of-frame κ genes and appreciating frequencies of nonfunctional κ mRNA, we show that these transcripts are all significantly down-regulated (≈4-fold) whether they belong to the VJ^{PTC} or the C^{PTC} class. This finding indicates that the conditions required for NMD or splicing inhibition of LC transcripts differ from those defined for other genes and that the occurrence of the PTC upstream (and at a distance) from a spliceable intron is not instrumental. This situation may be reminiscent from that of the viral *v-src* gene for which intronless transcripts are down-regulated (40).

Analysis of the rate of κ transcript functionality with regard to the Jκ use also shows that RNA surveillance is the less stringent for VκJκ2 (47.6% of functional transcripts). By contrast, it is the most efficient for VκJκ5 (85% of functional transcripts) and to a lesser extent for Jκ1 (72.4% of functional transcripts) (although nonfunctional mRNAs including Jκ1 are restricted to the C^{PTC} class). Whereas RNA surveillance of κ transcripts is unaffected by the position of the PTC upstream or downstream from an intron, this Jκ bias raises the question of a role for intronic sequences in mRNA down-regulation. The Jκ5 and Jκ1 3′ flanking introns may thus behave as better substrates than the Jκ2 3′ flank for a quality control process acting before or during splicing. By analogy, a portion of the T cell receptor Jβ–Cβ intron has been shown to stimulate mRNA degradation more efficiently than an isomerase gene intron (41).

Capillary electrophoresis allowed the study of down-regulation of out-of-frame antigen receptor transcripts and provided data in agreement with those obtained by sequencing pro-B cell genes and transcripts. It showed that out-of-frame primary κ transcripts were present in pro-B cells at a much higher rate than spliced transcripts, indicating that RNA surveillance occurred after or during splicing. However, there was no indication of an accumulation of nonfunctional unspliced transcripts and the NF/F ratio was not higher for primary RNA than at the DNA level. Capillary electrophoresis allowed the extension of our observations to multiple samples of normal mature B cells, all producing barely detectable levels of out-of-frame mature κ transcripts that contrasted with the higher amounts of out-of-frame κ genes and unspliced mRNA. Similar observations were made both in unsorted B cells including λ-expressing cells and in sorted κ-expressing cells. These data thus indicate that biallelic transcription of rearranged κ genes occurs in normal B cells. That out-of-frame Jκ1 junctions were more abundant among DNA sequences than among primary transcripts likely results from nonfunctionally rearranged Vκ–Jκ1 segments inverted by some secondary rearrangements. In contrast with Jκ5, which does not undergo such inversions by secondary rearrangement, NF/F ratios were roughly similar for DNA sequences and primary transcripts, suggesting that functional and nonfunctional κ alleles are transcribed at roughly similar rates. Culture in the presence of cycloheximide only resulted in a partial inhibition of the decay affecting aberrant κ transcripts from pro-B cells and in no detectable effect in normal B cells, suggesting that this surveillance pathway may be poorly dependent from translation. Rather than the translation-dependent NMD process, κ surveillance may principally rely on the inhibition of splicing, as previously reported for a V^{PTC} κ transcript (5).

This study documents κ-chain RNA surveillance in pro-B cells and mature B cells, showing that primary transcription of nonfunctional κ genes occur in normal B cells, whereas RNA surveillance down-regulates mature transcripts. This RNA surveillance pathway differs from classical NMD and can detect a frameshift without needing a PTC followed with an intron. That Jκ 3′ flanking introns are modulating this pathway suggests that frameshift detection occurs before or during splicing and then drives such transcripts toward degradation.

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